

Studies in the Experimental Analysis of Sex.

Part 11.—On Stylops and Stylopisation.

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With Plates 32-35.

It has long been known that the presence of Strepsipterous parasites on solitary bees and wasps may exert a remarkable influence on their hosts, and the paper by Professor J. Pérez in 1886 (1), gave a full account of the effect of *Stylops melittæ* on the bees *Andrena*, which must always be referred to as a classical contribution to the study of parasitic castration. Although many authors have subsequently written on these parasites, the observations of Monsieur Pérez remain unsurpassed for fulness of detail and interest, and we are indebted to the veteran entomologist for additional information which he has kindly put at our disposal in answer to inquiries. The re-examination of the subject, which is undertaken here, seemed desirable, not so much for the purpose of bringing new facts to light, but for confirming reported facts and collating them with the newly acquired results on parasitic castration brought about by *Sacculina*, since the effect of *Stylops* on its hymenopterous hosts seemed to be parallel to that of *Sacculina* on crabs,

and yet to differ from it in many important respects. The Strepsipterous parasites differ from *Sacculina* in that they have the sexes separate, whereas *Sacculina* is hermaphrodite, so that we can test whether the sex of the parasite has any influence on the effect excited. Moreover, it is clear that in certain cases the female host is induced to assume certain male characters as the result of parasitic castration by *Stylops*, an effect which is never observed in the case of *Sacculina*, and which requires careful examination. We have been able to study a large number of styloped bees of various species, but the most fruitful material consisted of a large colony of heavily styloped *Andrena nigroænea*, established in a grassy bank close to the University Museum, which was kept under observation for several years and afforded us a rich collection. The following observations on the structure and history of the parasite are based on the bees taken from this colony.

1. NOTES ON THE PARASITE.

Stylops melittæ, like all the Strepsiptera, has the sexes separate; the adult male being an extremely active-winged insect, showing a possible relationship to the Coleoptera (Pl. 33, fig. 8), while the adult female is a degenerate grub-like creature which remains permanently inside the body of the bee in which it is parasitic. The male, before hatching out as a winged insect, also develops inside the abdomen of the bee, undergoing its larval stages and pupation in this situation. The male pupa, in fact, closely resembles the adult female parasite, and protrudes a little cap between the segments of the bee's abdomen to the exterior, which closely resembles the head of the female parasite which is similarly protruded. When the adult-winged male emerges from its pupa and from the bee, it pushes off the protruded cap of the pupa and leaves the old empty pupal case inside the abdomen of the bee where it can often be recognised as a hollow cavity communicating with the exterior. Since the male *Stylops* emerge from their pupa soon after the bees

come out of their burrows for the first time in spring, the empty pupal case of the male *Stylops* is much more frequently met with in the bee than the pupa with its cap on containing the male itself. In the case of the female *Stylops*, it is quite different, since she remains permanently in the bee, and may be found all through the spring and early summer with her body distended with developing eggs or larvæ.

The appearance of a bee's abdomen with three female *Stylops* in it is shown on Pl. 32, fig. 1. The heads of the female *Stylops* are seen protruding between the segments of the abdomen, while the rest of their bodies are hidden inside the abdomen of the bee. If we dissect out the whole of the female parasite from the bee (Pl. 32, fig. 2), we see that its body consists of two chief parts, a hard yellow chitinous portion, the cephalo-thorax, which is protruded to the exterior, and a soft white segmented portion or abdomen, which is buried in the bee's abdomen, occupying a very large proportion of the hæmocoel or body cavity of the bee.

There was for a long time some doubt as to whether the hard protruded chitinous part was really the head or the tail end of the *Stylops*, but it is now quite certain that it is really the head end, as it is possible to prove by means of a median sagittal section, such as is shown on Pl. 32, fig. 3, that the brain (*gn. 1*) and the subœsophageal ganglion (*gn. 2*) are situated there. We can also establish from the position of the ganglia and the ventral nerve cord (*n*), that the surface of the cephalo-thorax exposed to view when the parasite is *in situ* on the bee is the ventral surface.

Looking at this ventral surface of the cephalo-thorax we can see (Pl. 32, fig. 2), certain tubercles and slits which are of importance. A median tubercle near the extreme anterior end is placed just behind a minute pore which represents the mouth (*m*). On each side of the mouth are a pair of lateral tubercles which probably represent the atrophied mandibles (*md*). Behind these tubercles is a bow-shaped slit (*o*), the opening of the brood passage, by which the larvæ are

finally liberated. This slit at maturity communicates with a superficially-placed passage running all along the ventral surface of the body. The lateral limits of this passage are indicated by the ridges (*r*) shown in the figure. The relations of this brood passage are shown in the sections on Pl. 32, figs. 3 and 4 (*b*). It will be seen that the passage is simply a space between the chitinous integument and the body wall, the epithelium of which (*ep*) is peculiarly modified by the cells being produced into spiny processes. The function of this curiously modified epithelium of the brood-passage has never been suggested, but possibly its rough surface is convenient for the young larvæ to travel over in their journey to the exit at *o*.

The eggs complete their development in the body of the female, which at maturity consists of a mere sack containing them, all the other organs being reduced to vestiges. An idea of the appearance of a fully ripe female with the body full of active larvæ and embryos is given by the photograph on Pl. 33, fig. 6. The fully-developed larvæ reach the brood passage from the body cavity of the parent by means of five peculiar trumpet-like invaginations which lead into the brood passage and acquire openings into the body of the parent at the moment that the larvæ are ready to escape. The five trumpets are shown attached to the epithelium of the brood passage, the rest of the body having been removed, in the photograph on Pl. 33, fig. 7, and a trumpet with its opening into the brood passage is shown in the sections on Pl. 32, figs. 3 and 4.

We may make some mention of the other organs of the body of the female parasite. The skin, except where the epithelium of the brood passage is specially modified, is exceedingly thin, and the nourishment of the body must take place by absorption through this thin skin. There are no special cells for seizing on or elaborating a special kind of food either in the skin or elsewhere, so that we may suppose that the hæmolymp of the bee affords a ready-made medium which supplies the parasite with all that is requisite. This is in

perhaps significant contrast to *Sacculina*, where a special and highly-developed system of roots ramifies through the body of the host, and is engaged in seizing on a special constituent of the food, viz. fat.

The alimentary canal (Pl. 32, figs. 3 and 4, *g*), of the *Stylops* is clearly recognisable, but in a quite degenerate and useless state. There is a minute mouth opening (*m*), and an equally minute anus at the hind extremity, but the lumen of the gut through the body is obliterated. The whole apparatus is obviously functionless. There is a peculiar mass of cells with dark staining nuclei and eosinophilous cytoplasm (Pl. 32, fig. 3, *c*) situated in the ventral part of the cephalo-thorax; the function of these cells is unknown, but possibly they are of the nature of supporting-cells analogous to cartilage, to stiffen the exposed region of the body. Dorsally to the gut the remains of the dorsal blood-vessel or heart (Pl. 32, figs. 3 and 4) can be recognised. The nervous system consists of three ganglionic masses (Pl. 32, fig. 3, *gn* 1, 2, and 3), the dorsally-situated ganglion, (*gn* 1), being connected by means of a thin commissure round the œsophagus with a large ganglion in the thoracic part of the cephalo-thorax (*gn* 2). From this ganglion a thin, nervous strand (*n*), representing the ventral nerve cord, passes to a third ganglion (*gn* 3) in the abdomen, and from this a thin filament passes away, but no other ganglia could be found in the adult female.

Of great importance for the economy of the parasite is the tracheal system. There are two main tracheal trunks which open on conspicuous tubercles (Pl. 32, fig. 2, *tr.*) on each side of the cephalo-thorax. These two tracheæ (see Pl. 32 and 33, figs. 4 and 7, *tr.*) pass right through the body, giving off numerous branches, which ramify among the developing eggs and supply them with oxygen from the outside, quite independently of the bee. We may note again a contrast between *Stylops* and *Sacculina* here, since the developing *Sacculina* roots have to obtain their oxygen from the blood of the crab.

The above account of the structure of the female parasite

is sufficient to give an idea of its mode of life and nourishment; for further details the reader may refer to the papers of v. Siebold (2), Pierce (11) Nassonow (10) and Brues (8). There are certain points relative to the life-history of the parasite which remain obscure but suggest features of great interest. It is known that the larvæ, which are called *Triungulins* and have the form shown in ventral view on Pl. 32, fig. 5, leave the body of the parent by means of the opening of the brood passage, and find their way on to flowers visited by the bee. They then clamber on to another bee which visits the flower, and clinging on to its hairs are carried back to the burrow, where they ultimately enter the cells and infect the next generation. At exactly what stage they enter the young bee larvæ is not known, but it is presumably at an early stage of development.

The really obscure part of the life-history concerns the mode of development of the eggs, and the question, how the female parasite is fertilised by the male, if, indeed, fertilisation ever takes place.

In our account of the structure of the adult female parasite it was shown that the portion of the body protruded to the exterior is the head, and not the tail, as certain authors have supposed. Apart from the anomaly of fertilisation taking place through the head end of an insect, we have been unable to find any opening or organ such as a spermatheca for the entrance or reception of the spermatozoa of the male, either on the protruded cephalo-thorax or on the rest of the body inside the bee. It has been suggested by some authors (16) that fertilisation may take place through the opening of the brood passage, but this appears to us improbable, as there is no means of entrance from the brood passage into the body of the parasite, the trumpet-like invaginations being completely closed until shortly before the larvæ are ready to emerge. It has also been suggested that fertilisation might take place through the mouth and alimentary canal, but this rather extravagant suggestion is not supported by the actual state of the alimentary canal, which appears in sections as a con-

tinuous narrow tube passing through the body without any outlet into the body cavity where the eggs are contained.

It has already been suggested that the eggs of Strepsiptera may develop parthenogenetically in certain cases. Thus Brues (8), describing the oogenesis in *Acrochismus wheeleri* (Pierce), a Xenid parasite on the wasps *Polistes*, contends that the eggs develop parthenogenetically, the second polar body re-entering the egg and fusing with the egg nucleus. Since Brues, however, did not follow polar-body formation his evidence is incomplete.

Dr. R. C. L. Perkins (5) has expressed the view that the parasites of the bee *Halictus* must be parthenogenetic, at least in certain cases, as when the eggs begin to develop it is impossible that a male could have fertilised the female. Dr. Perkins also has kindly informed us that out of 500–1000 specimens of this parasite seen by him only one or two were males, so that evidently the males are on the point of disappearance. Taking these facts in conjunction with the practical impossibility, as it seems to us, of the spermatozoa in *Stylops* ever entering the body of the female at the time the eggs begin to develop, we are led to the conclusion that development is always parthenogenetic in the *Stylopidæ*. If this is correct, it follows that the active winged males are useless for the propagation of the species, a conclusion which few would accept without misgiving.

Various observers have attempted to observe the act of copulation in the Strepsiptera, but mostly with no or very equivocal success. Pierce (11), in his valuable revision of the Strepsiptera, remarks—"That the female must be fertilised can hardly be doubted, and yet the nature of the act and the fact itself has been but slightly proven." Observations on the behaviour of the male by Saunders and Crawford (11) show that he is actively attracted by the female *Stylops*, or, at any rate, by the bee on which a female *Stylops* is situated, and that he runs about on the body of the bee, evincing the greatest excitement. F. Muir (17) has made similar observations, but is uncertain how copulation takes place.

The following account, compiled by one of us (A. H.) from notes on the habits of the male, may be given :

At the end of April and beginning of May, 1912, the male *Stylops* was not uncommon in the vicinity of the colony of *Andrena nigroænea*, being seen on the wing at mid-day in sunshiny weather. The singular flight of the male *Stylops* has often been seen and commented upon by many observers. When once recognised they can never be forgotten, the peculiar flight and milky-white wings at once distinguishing them from all other insects. None were observed actually flying over the burrows of the bee, and nearly all, when first seen, were some 10 or 15 feet from the ground, sufficiently high to prevent some of them being captured. All the specimens caught were boxed alive for further observation or experiment. On three occasions a male *Stylops* was, immediately after capture, introduced into a large glass-bottomed box containing a freshly caught bee, infected with one or more female *Stylops*. In each case the behaviour of the male was identically the same. The male *Stylops*, directly it was introduced into the box, fluttered on to the bee, and quickly ran over its body to where the head of the female *Stylops* was everted between the bee's abdominal segments. At this time the male is rapidly vibrating its wings and protruding its last two or three apical segments, which are long and tapering like an ovipositor. The insect is thus quite unlike a dried specimen in which these segments are invariably telescoped into the body. Actual pairing did not occur on any of the three occasions. The bee, which had been resting quietly in the box, became extremely restless as soon as the male *Stylops* flew on to it, and kept repeatedly climbing to the top of the box and then suddenly dropping, as if in the endeavour to rid itself of its unwelcome rider. After about ten or fifteen minutes of ceaseless running to and fro over the bee, the male *Stylops* voluntarily quitted the *Andrena*, but still continued to run and vibrate its wings for about two hours longer, after which time it dropped apparently exhausted, and died shortly afterwards. The other males which had

been boxed also continued to flutter and to vibrate their wings ceaselessly, until, in about two hours, all movement came to an end, and they were apparently dead. It will be seen from this account that the male *Stylops*, besides retaining its structure and activity unimpaired, also possesses the instinct for attaching itself to the bee, but in no case has actual copulation been observed.

The copulatory apparatus of the male *Stylops* (fig. 8, A and B) consists of a hollow chitinous penis, shaped rather like a pick-axe, which can be everted on a hinge, but when withdrawn is covered by a grooved sheath. Although the penis is a slender organ it has a sharp point, and might be used for hypodermic injection of spermatozoa into the body of the female. It is, however, very difficult to see how the injection of spermatozoa into the body of the female would result in fertilisation, because the eggs never quit the ovary, and are always completely surrounded by follicular epithelium, which would prevent the access of spermatozoa casually injected into the body-cavity. The male does not show any trace of degeneracy in its internal reproductive organs, the vesiculæ seminales being crowded with active spermatozoa.

In several females the eggs have been found in an early stage of development, the features of which strongly confirm our suspicion that development is parthenogenetic. In these cases all the developing eggs are at approximately the same stage of development, exhibiting two, or, in some cases, more segmentation nuclei (Pl. 32, fig. 4 A, *bl.*), while at the periphery of the egg a mitotic spindle is observed (p. 1), which invariably exhibits a single large chromosome and three, or four smaller ones, often in process of division. Each egg is completely invested by the follicular epithelium (*f*).

Now, it is quite clear that the mitotic spindle must represent the first polar body in process of division. There is, however, no trace of a second polar body, which there certainly ought to be if a second polar body was given off and fertilisation effected in the usual way.

It is possible to explain the appearances in these eggs on three suppositions: (1) That no second polar body is formed and that the female pronucleus develops parthenogenetically, (2) that a second polar body is formed but fuses with the female pronucleus, which then develops parthenogenetically, or (3) that a second polar body is formed but disappears and leaves no trace, although the first polar body is still only in the metaphase of its mitosis, and that fertilisation is effected by a spermatozoon.

It must be admitted that the third supposition is exceedingly improbable from what we know of the development of any other egg, the entire disappearance of the second polar body before the completion of the mitosis of the first polar body being altogether unknown.

During the present year (1914) the burrows of the colony of *A. nigroænea* were carefully watched at the beginning of the season, and the first bee carrying a female *Stylops* was captured, and the parasite preserved and its eggs examined by serial sections. These eggs were found to be already in a fairly late segmentation stage, which is strong presumptive evidence that development had already begun before the bee had left its burrow, and before the *Stylops* would have had a chance of being fertilised.

It will therefore be seen that the cumulative evidences in favour of the parthenogenetic development of the eggs of *Stylops* are exceedingly strong, consisting in the following main heads: (1) There is no opening or apparatus in the female adapted for conveying the spermatozoa to the eggs; (2) the eggs remain throughout their development encased in the follicular epithelium of the ovary, so that access to them by spermatozoa which had entered the body cavity is very difficult to imagine; (3) parthenogenesis must occur as a normal rule in the parasites of *Halictus*; (4) the known stages in the polar-body formation of *Stylops* are inconsistent with the view that fertilisation by a spermatozoon has been effected; (5) actual copulation by the male has never been adequately observed.

We may finally note that in a large number of colonies of infected *Andrena* it would appear that the male parasite is very much scarcer than the female, and in certain cases may have almost entirely died out. This rule is, however, not invariable, and in the case of *Xenos*, Wheeler (12) records an actual preponderance of males over females.

The most difficult thing to explain, on the assumption that the males are now useless, is their marked instinct for clambering on the body of the bee when infected by a female *Stylops*. This surely indicates that at some time pairing took place in this situation. In explanation of this it must be remembered that under present conditions the female *Stylops* never develops beyond what is really a larval or rather pupal stage, and that at some previous period in the history of the parasite it is certain that the female developed further and probably issued from the bee as a fully-formed and possibly winged imago. It may well have been that when this was the case the males waited for the emergence of the females and paired with them directly they issued from the bee, and that they still retain the instinct of attaching themselves to the infected bees and waiting for the appearance of the female imago, an appearance which now is never realised. For an explanation we are forced to fall back upon some such hypothesis as this, since no transitional forms are known to exist in nature which might show the intermediate steps by which the endo-parasitic habit and arrested development of the female parasite have been acquired.

If we are correct in supposing that the males of the *Stylopidæ* are useless and that development is invariably parthenogenetic, it may be pointed out that such a condition of affairs is not altogether without parallel according to the results of recent researches. In the *Rhizocephala* (13) degenerate *Cirripedes* parasitic on various Decapod crustacea, we find that in certain genera, e.g. *Sacculina* and *Peltogaster*, the parasites are hermaphrodites which propagate themselves by a continuous round of self-fertilization. Nevertheless, degenerate larval males are found, often in

numbers up to twenty or thirty, fixed round the mantle opening of about 80 per cent. of young *Sacculinæ*, and these larval males are entirely degenerate and useless in the reproduction of the species. Other genera of the *Rhizocephala*, e. g. *Sylon*, are purely female, and reproduce entirely by parthenogenesis, and have thus got rid of the marked disharmony (to borrow Metchnikoff's term) which characterises the sexual economy of *Sacculina* and *Peltogaster*. Another instance of sexual disharmony has been described by Maupas in certain free-living *Nematodes* of the genus *Rhabditis*, where again the majority of the individuals are self-fertilizing hermaphrodites, but a certain number of males are still produced which are useless for reproduction. In other species these males have been entirely eliminated.

These instances of undoubted disharmony, or imperfection of adaptation in sexual economy, should make us pause before we assume that the males of the *Stylopidæ* must still be functional in those species in which they occur in considerable numbers.

2. THE EFFECT OF THE PARASITES ON THEIR HOSTS.

We will consider first the effect of the parasites on the internal reproductive organs of the hosts.

In the case of twenty female *Andrena nigroænea*, of which four carried male *Stylops* puparia and sixteen female *Stylops*, it was found in every case that the ovary was very greatly reduced in size and was incapable of producing mature ova. The appearance of such reduced ovaries, as compared with that of normal ovaries, is shown on Pl. 33, figs. 9 and 10.

No marked difference was observed in cases where male parasites were present from those in which female parasites were concerned.

It was found, therefore, without exception, that stylopisation brought about a reduction in size of the ovary and complete sterility.

This result is in agreement with the observations of Pérez (1).

In the case of fifteen male *A. nigroænea*, of which four carried male puparia, ten female Stylops, and one had a male and a female parasite, it could not be observed that the presence of the parasites in any case had exerted any effect on the development of the testes or their ducts. The figures given on Pl. 33, figs. 11 and 12, show the male reproductive apparatus in normal and styloped males, and it will be seen that there is no reduction in size in the styloped individual. In order to test whether the testes of the styloped males produce ripe spermatozoa, it was found necessary to examine bees early in the year soon after their emergence from the burrows, since both normal and styloped individuals later in the year were generally found with the vesiculæ empty of spermatozoa.

If, however, styloped males are taken early in the year, it is possible to show that their testes and ducts are in the same condition as normal males, and that abundance of ripe spermatozoa are present in the large vesicles which lead from the three testicular tubes on each side into the vas deferens. The section on Pl. 33, fig. 13, through the three testicular tubes and the vesicle of one side of a styloped bee, shows the presence of abundant spermatozoa in the vesicle. The testicular tubes in this section are more or less empty with a rather ragged degenerate epithelium, but this appearance is due to the fact that spermatogenesis is over, and is equally to be noticed in normal males.

This absence of any effect of the stylopisation on the male internal organs is on the whole in agreement with what other authors have found, though Pérez records some cases of a one-sided damage being inflicted on the testes by the parasite, and Theobald (7) is inclined to believe that the damage may be considerable. Perkins (5), on the other hand, both for males and females, tends to minimise the effect of the parasites on the internal organs, and records the fact that styloped males have been taken in copula with

styloped females, showing that the sexual instincts may still persist in styloped individuals.

There can be no doubt from our own observations, and from the general consensus of opinion, that the female bees suffer far more serious reduction in their ovaries than the male bees experience in the case of their internal reproductive organs.

The reason for this difference in effect appears to us fairly obvious. The testes of the male bee are exceedingly minute structures, about a hundredth part of the ovaries in size, and they, therefore, require a small fraction of the nutriment which is demanded by the ovaries. The presence of the parasite, therefore, while cutting off a large part of the necessary nutriment from the ovaries, does not succeed in depriving the minute testes of the small amount of nourishment which they require, and hence they are able to attain their normal development, though the ovaries are seriously starved.

We may now proceed to the effect on the external characters.

The males and females of *A. nigroænea* differ, firstly from one another in their external genital armature which consists of a complicated copulatory apparatus in the males and of an ovipositor in the female. After examining a long series of styloped males and females, we are unable to find any reduction or abnormality in these structures as the result of stylopisation. In this respect we are not in complete agreement with Pérez (1), who reports a marked reduction in the development of the genital armature as the result of stylopisation in several cases. We do not doubt that this is correct, but the effect in any case is a comparatively slight one, and there is never the slightest difficulty in at once recognising the male and female styloped individuals by the genital armature which is always typically developed, though it may be in certain cases somewhat reduced in size.

Another important secondary sexual character, affecting the hard chitinous structure of the bee is found in the

antennæ, which are 13-jointed in the males and 12-jointed in the females, of all *Andrena* (see Pl. 33, figs. 14-15).

This character in our experience and in the experience of all other observers is quite unaffected by stylopisation, the infected individuals always having the number of joints typical of their sex. Pérez again is of opinion that in certain cases the relative length of the joints in infected individuals is slightly altered, but here the effect is admittedly very slight indeed, and the figures given to illustrate the effect do not appear to us to bear out the contention.

A marked distinction between the sexes of all *Andrena* is found in the structure of the femur and tibia of the hind legs, which are thin and not markedly hairy in the male, but in the female are greatly enlarged to form the scopa or pollen-collecting apparatus. The condition of the normal male (*A. nigroænea*) is shown on Pl. 34, fig. 16, of the infected male, in Pl. 34, fig. 17, of the normal female in figs. 18 and 19, and of two stylopised females in figs. 20 and 21.

These figures bring out the fact, which we have found invariably in *A. nigroænea*, that as the result of stylopisation the male does not acquire in any degree the scopa of the female, while the scopa of the female is always to some extent reduced in size by the action of stylopisation.

We also find that stylopised females never carry any pollen on their scopæ, in marked distinction from the normal females, the majority of which are found with their scopæ plastered with pollen as shown in fig. 18. The stylopised females have evidently entirely lost the instinct for collecting pollen, though they still continue to visit the burrows. Of the hundred or so stylopised females examined not a single individual had pollen on it, but we are not in a position to say that such a thing cannot ever occur, as there is certainly a great degree of variation in the intensity of the effects of stylopisation in different individuals and species.

We have found that stylopisation affects the punctuation of the chitin of the abdomen to a certain extent, though the effect can only be appreciated by examining a good series of

normal and infected individuals together. If we look at a series of normal males and females together, we shall notice that the males reflect the light more brightly than the females, owing chiefly to the less degree of punctuation and hairiness of the abdomen.

The styloped males, on the other hand, tend to have the abdomen dull, very much as in the female, and this appears to be due to the deeper and more frequent punctuation on the abdomen, and not to a greater hairiness. The styloped females do not appear to be affected either in punctuation or hairiness.

We have now dealt with the most important secondary sexual characters which concern the structure of the hard chitinous parts, and it will be recognised that the effect exerted by stylopedisation is small and consists in a reduction of certain sexual characters, and never in a real assumption of characters proper to the opposite sex. The most constant and striking effect is the reduction of the scopa in the female and the loss of the instinct for collecting pollen. Comparing these effects with the effect of *Sacculina* on the secondary sexual characters of *Inachus* (14), it will be admitted that the complete inversion suffered by the males of *Inachus* has no parallel in the bees modified by stylopedisation, so far as structure is concerned.

There remains for consideration, however, a very important character which may undergo a very complete inversion as the result of stylopedisation. In certain species of *Andrena*, e. g. *A. chrysoceles* and *A. labialis*, the female has the ordinary black clypeus, but the male has a yellow or white one (see Pl. 35, figs. 22, 23, 25 and 27). Pérez discovered that as the result of stylopedisation the female might assume completely or in part the coloured clypeus of the male (see Pl. 35, fig. 26), while the male might undergo considerable retrogression and lose a great part of the yellow colouration (fig. 28). Pérez makes it clear and has personally informed us that this remarkable effect is by no means invariable and that very frequently styloped males and females of *A.*

labialis may exhibit their proper clypeus colouration without any modification. It is therefore necessary in this case to be able to examine a long series of infected individuals, and it would appear that other observers have not been fortunate in securing such a series because no confirmation of Monsieur Pérez's discovery has hitherto been published. We are fortunately in a position to produce confirmatory evidence in the case of *A. chrysoceles*. Pl. 35, fig. 22, depicts the head of a normal female specimen, while fig. 23 shows the head of a normal male with the coloured clypeus. Fig. 24 shows the head of a styloped female, taken at Sandford near Oxford by one of us (A. H. H.), which has developed the coloured clypeus of the male in a typical manner. This specimen was parasitised by a male *Stylops*. It may be stated that long series of normal *A. labialis* and *chrysoceles* have been examined, and that in no case has any assumption of the coloured clypeus by the female or *vice versa* been observed apart from the effects of stylopedisation.

This acquisition of clypeus colouration by the female is by far the most striking alteration brought about by stylopedisation, because it really amounts to a true acquisition of a positive character belonging to the opposite sex, and not to a mere negative suppression of characters that should normally be developed. The alterations in the hard parts and the blackening of the clypeus in the males, can all be interpreted as mere negative suppressions, but the acquisition of the yellow clypeus by the styloped females is in a different category. In the majority of *Andrena* the clypeus of both sexes is black, so that the loss of the yellow colour in the styloped males of *A. labialis* may be considered a mere repression, but not so the acquisition of the yellow colour by the female.

Before discussing these results there are two points which merit attention. Since the *Stylops* parasites are of separate sexes, it appeared possible that the sex of the parasite might have an important influence on the effect exerted upon the bee. For instance it might be found that

only females styloped by male Stylops could develop the white clypeus characteristic of the normal male bee. Of course if such a contention could be proved it would have a most important bearing on the theoretical interpretation of how the effect is brought about. It would suggest, in fact, that the male Stylops exerted a specific male influence on the bee, and the female Stylops a specific female influence.

In answer to our inquiries Monsieur J. Pérez has kindly sent us some of his specimens of *A. labialis*, which satisfactorily settle this point. Among these specimens there are two female *A. labialis* parasitised by female Stylops which show a considerable amount of white colour on the clypeus, and there is also a male *A. labialis* parasitised by a male Stylops, which shows a very marked reduction of the white colour on the face.

Dr. R. C. L. Perkins has also sent us two valuable instances bearing on this question, viz., two females of *A. labialis*, with their faces coloured as in the male, both of which are parasitised by a single female Stylops. These instances are abundantly sufficient to demolish the view that the sex of the parasite has any determining influence on the effect produced on the secondary sexual characters. It is probably true that the presence of a male Stylops has a more generally damaging effect on the bee, but there is no evidence of the male parasite exciting a specifically male effect and of the female exciting a female effect upon the host.

The theoretical importance of this fact will be given its due weight in a later paragraph.

The second point to which attention may be called is the great amount of variation exhibited by *Andrena* and other insects in their reaction to strepsipterous parasites. This variation does not only subsist as between different species of hosts, but also as between different individuals of the same species of host. Wheeler (12), who has made a most detailed and exhaustive examination of the effect of *Xenos* on the wasps *Polistes* came to the conclusion that the parasite had no definite effect on the secondary sexual characters of the

host, and it is clear that his conclusion is perfectly correct. The same may probably be said of the effect of *Elenchus* on the Homoptera.

We have already seen that no other observer has apparently described the effect of *Stylops* on the clypeus colouration of certain *Andrena*, noticed by Pérez, until we came across the case of *A. chrysoseles* published here. These apparently contradictory results have led to much confusion, but to anyone familiar with the facts of parasite castration in other branches of the animal kingdom they will occasion no surprise. In cases where the effects of the parasite lead to the most startling and complete inversions, as in *Sacculina* on *Inachus* and *Peltogaster* on *Eupagurus* (14), there is always a certain small percentage of individuals which remain almost completely unaffected by the presence of the parasite, while in other cases, such as *Sacculina* on *Carcinus*, the effect of the secondary characters is often nil, and never consists in more than a slight approximation of the male to the female type. Such variations then, whether due to differences in individual or specific susceptibility or to some casual event in the history of the disease, are to be expected, and should warn the observer not to draw conclusions without examining a long series of infected individuals.

(3) DISCUSSION OF RESULTS.

If we compare the effects of stylopisation with those of *Sacculina* on *Inachus*, we shall recognise that they are much slighter and less radical in the former than in the latter case. Thus in the internal reproductive organs stylopisation only causes a reduction in size of the ovary, and prevents ripe ova from being produced, while the testes of the male are practically unaffected. The presence of *Sacculina*, on the other hand (13), may occasion the complete destruction of all the internal genital organs, with the exception of some remnants of germinal epithelium, while infected

male crabs may be induced by the *Sacculina* to produce ripe ova in their testes.

Correspondingly the effects of stylopisation on the secondary sexual characters are comparatively slight, and amount to no more than a reduction of certain characters, such as the scopa of the female, while in the case of *Sacculina* the whole morphological structure of the male crab may be entirely converted to the female state.

In one case, however, that of the colour of the clypeus, the female bee when stylopised may assume the positive male character.

It is clear, therefore, that the reaction of the bee to the *Stylops* does not go so far as that of the crab to *Sacculina*, either internally or externally, and whereas in sacculinisation we are forced to the conclusion that the *Sacculina* exerts an active feminising influence on both sexes of infected crabs, in the case of stylopisation it is sufficient to hold that the action here consists merely in an arrest of development incident on the cutting off of a certain amount of nutriment from the ovaries, and to a less extent from the testes. In sacculinisation we have argued (13) that the *Sacculina* roots, by demanding a certain type of nutriment, viz. fat, stimulate a certain type of metabolism in the crab, which is characteristic of the adult female when maturing its ovaries, and that this internal change of metabolism brings in its train all the deep-seated changes in the internal and external genital structures. The *Stylops*, on the other hand, does not initiate such wide-spreading changes; it stops short at abstracting a certain amount of nutriment from the blood, and so causes a merely quantitative alteration in the development of the internal and external genital organs. It has been pointed out that the *Stylops* parasite does not appear to be taking up any special nutriment from the blood of the host, but rather to receive the nutriment from the blood, ready-made, and thus it would not be expected to stimulate any special line of metabolic changes. Further, the *Stylops* always receives its oxygen from the outside air, while the

Sacculina roots are living anaerobically, and must split off their oxygen from the blood of the host. This implies a more intimate relation between the metabolism of host and parasite in the case of *Sacculina*.

We thus see that the effects of stylopisation may be interpreted as due to a merely quantitative abstraction of nutriment normally destined for the reproductive glands, and that this abstraction brings in its train a reduction in size of these glands, especially the ovaries, and a corresponding reduction in the development of some of the secondary sexual characters.

It may be urged, however, that this explanation does not apply to the assumption of the yellow clypeus by the female, as this is a positive male character. We have an excellent analogy for this case in sterile female birds (15) which, either as the result of operative ovariectomy or else of ovarian disease and atrophy, may assume male plumage to a very marked extent. The assumption of the yellow clypeus by stylopised female bees with reduced ovaries seems to us exactly parallel to the assumption of cock's plumage by female birds with ovaries either atrophied or removed by operation. It seems that in both cases the mere atrophy or suppression of the ovary is sufficient in both cases to induce the development of certain male characters in the colouration.

It is not necessary, therefore, to ascribe a special masculinising influence to the *Stylops* parasite in the same sense as one must ascribe an active feminising influence to the *Sacculina* roots. The masculinising influence resides in the female bee itself, just as in the female bird, and is called into activity by the mere suppression of the ovarian function.

In this manner we may look upon the acquisition of the yellow clypeus by the female as due to the same cause as the other alterations brought about by stylopisation, viz. to the mere quantitative cutting off of nutriment from the ovary, and not to any specific or qualitative action of the parasite, as in the case of *Sacculina* on *Inachus*.

It appeared possible to us at one time that a qualitative action might account for the assumption by the female of the male clypens in the following manner: It might be possible that this assumption by the female only followed when she was parasitised by a male *Stylops*, which might exert a specific male influence on the host. This supposition is not confirmed by the facts, as the presence of a female *Stylops* can equally bring about the assumption of the male clypens by the female.

The fact that the sex of the parasite has no influence on the effect exerted on the host is in reality a strong confutation of the idea that the effects of parasitic castration are due to a specific internal secretion produced by the parasite. For if such a secretion were produced by the parasite, we should certainly expect that the female parasite would produce a female internal secretion and the male a male one, whereas we find that the parasites of both sex exert a similar effect. This is perfectly intelligible if we suppose that the parasites of both sexes act on the host merely by cutting off a certain amount of nutriment from the gonad, a process which reacts more profoundly on the female than on the male, owing to the larger size of the ovaries and the larger demand made by them on the nutriment in the body.

The peculiarity of the case of *Sacculina* consists in the fact that the roots of the parasite happen to demand an excessive supply of the same sort of nutriment which the ovary of a normal female crab requires, and so bring about a series of profound metabolic changes leading to the feminisation of the host.

The result of all the above considerations is to show that a parasite may act on the sexual characters of its host in two ways. Firstly, it may simply take up a certain amount of nutriment from the blood so as to deprive the gonad of its proper supply and lead to its partial atrophy, but without bringing about any deep-seated alteration in the metabolism or stimulating any special set of metabolic changes. The abstraction of this nutriment, by depriving the blood of its

proper supply, may lead to the atrophy of the gonad and to the reduction of the secondary sexual characters, and even to an inversion of certain secondary sexual characters, e. g. the colour of the clypeus in *Andrena*. Secondly, as in the case of *Inachus* parasitised by *Sacculina* and of *Eupagurus* parasitised by *Peltogaster*, the parasite in obtaining its food from the blood of the host, may set going a special set of metabolic changes in the host, and these changes may result in diverting the metabolism of the host to the female state, so that the host assumes female characters throughout and the infected male may even be induced to produce ova in its testes. This second type is not a mere passive inhibition like the first, but an active reaction explicable, as has been shown, on the basis of an immunity reaction (14).

Another point which emerges from this study is that the bee, *Andrena*, belongs to the same category as many birds (Pheasants, Fowls, Ducks, Ostriches, etc.), (15) in that a mere atrophy of the ovary is followed by an assumption of a positive male secondary sexual character. In all these animals it would appear that the normal ovary exerts an inhibitory action preventing certain male characters from emerging, and that when the ovarian influence is removed or interfered with the stimulus is given for the development of these characters.

SUMMARY.

(1) From a study of the anatomy and life history of *Stylops*, it appears that despite the existence of active winged males, fertilisation cannot occur and development is always parthenogenetic.

(2) The parasite obtains its oxygen from the outside air by means of tracheal openings on the cephalo-thorax, and it does not possess any special absorptive organs for taking up a special kind of food from the host. Nutrition appears to take place by simple filtration from the host's blood through the very thin skin of the parasite.

(3) The effect of the parasite on the internal genital organs is slight, as compared with the effect of *Sacculina* on *Inachus*, and leads to a reduction in the size of the ovaries to about quarter the normal size, while the testes are usually unaffected. The ovaries of stylopised bees never produce ripe ova, but the testes generally produce normal ripe spermatozoa.

(4) The effect on the secondary sexual characters is again slight as compared with that of *Sacculina* on *Inachus*. The external gonapophyses are usually unaltered, or they may be slightly reduced in size; the antennæ are unaltered. The scopa of the parasitised female is generally reduced in size, and she never or very rarely collects any pollen. The punctuation on the abdomen of the male may be increased.

(5) The most striking effect occurs in certain species (e. g. *A. labialis* and *chrysoceles*) in which the male normally has a yellow clypeus and the female a black one. Stylopisation in those cases may lead to the female assuming a yellow clypeus as in the male, while the male may lose the yellow and acquire a partially black clypeus.

This acquisition of the yellow clypeus by the female is the only change which can undoubtedly be interpreted as a positive acquisition of a secondary sexual character proper to the opposite sex.

(6) This effect may be brought about by male or female *Stylops* indifferently, the sex of the parasite having nothing to do with the nature of the effect exerted.

(7) The effects of stylopisation may be ascribed to a merely quantitative abstraction of nutriment from the gonad, leading to its partial atrophy, and not to a qualitative alteration of the metabolism such as is brought about by *Sacculina*. This also applies to the assumption of the yellow clypeus by stylopised females, on the analogy of the assumption of male plumage by many female birds as the result of simple ovariectomy or ovarian atrophy.

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